CHARACTERIZATION OF EXTRACELLULAR PROTEASES FROM *PSEUDOMONAS* SP. 16D4 AND *DERMACOCCUS* SP. 17D6 ISOLATED FROM ARCTIC REGIONS

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ABSTRACT

In recent studies, cold-active enzyme has become another subject of interest. It can overcome the limitations of the classical industrial enzymes that normally require higher temperature to function. In this study, 16 bacteria isolated from the Arctic region were screened for their protease activity using Skim Milk agar plate. Two bacteria with lower hydrolysis coefficient (Hc) were selected, they were *Pseudomonas* sp. 16D4 (Hc = 0.339) and *Dermacoccus* sp. 17D6 (Hc = 0.331), and their proteases were produced using "Protease Specific Medium" and were further studied. Both bacteria produced proteases that are associated to growth and the maximal protease activity was reached at 30th hour and 24th hours of incubation, respectively. Protease produced by *Pseudomonas* sp. 16D4 remained stable at temperature ranging from 0^{o}C to 70^{o}C. Its optimum activity was detected at 0^{o}C, pH 9 or 11 with K_m value of 1.37 mg/mL and V_{max} value of 243.90 Unit/L. As for *Dermacoccus* sp. 17D6, the protease activity remained stable at temperature ranging from 0^{o}C to 40^{o}C and its optimum condition for the assay was at 50^{o}C, pH 7 or 9 with K_m value of 0.66 mg/mL and V_{max} value of 344.83 Unit/L. The SDS-PAGE and zymogram analysis further revealed that the molecular mass of the *Pseudomonas* sp. 16D4's protease was 40 kDa. Whereas the size for the *Dermacoccus* sp. 17D6's protease cannot be determined due to the negative result obtained in the zymogram analysis.

ABSTRAK

Enzim sejuk-aktif telah menjadi satu lagi subjek yang menarik pada zaman ini. Ia dapat mengatasi batasan enzim perindustrian klasik yang biasanya memerlukan suhu yang lebih tinggi untuk berfungsi. Dalam kajian ini, aktiviti protease bagi 16 bakteria dari kawasan Artik telah disaring dengan kegunaan Skim susu agar. Dua bakteria dengan pekali hidrolisis (Hc) yang lebih rendah telah dipilih, mereka adalah *Pseudomonas* sp. 16D4 (Hc = 0.339) dan *Dermacoccus* sp. 17D6 (Hc = 0.331). Dengan kegunaan "Protease Specific Medium", protease mereka telah dihasilkan dan digunakan dalam kajian seterusnya. Kedua-dua bakteria menghasil protease yang berkaitan dengan pertumbuhan dan maksimum protease aktiviti boleh dicapai pada jam 30 dan jam 24, masing-masing. Protease yang dihasilkan oleh *Pseudomonas* sp. 16D4 dapat mengekalkan kestabilannya dari suhu 0^oC hingga 70^oC. Aktiviti optimumnya pula dikesan pada 0^oC, pH 9 atau 11 dengan nilai K_m = 1.37 mg/mL dan nilai V_{max} = 243,90 Unit / L. Bagi *Dermacoccus* sp. 17D6 pula, enzimnya dapat mengekalkan kestabilan pada suhu antara 0^oC hingga 40^oC dan keadaan optimum untuk enzim itu adalah 50^oC, pH 7 atau 9 dengan nilai K_m = 0.66 mg mL dan nilai V_{max} = 344,83 Unit / L. Analisis SDS-PAGE dan zymogram telah mendedahkan bahawa molekul saiz bagi enzim *Pseudomonas* sp. 16D4 adalah 40 kDa. Bagi enzim *Dermacoccus* sp. 17D6 pula, saiznya tidak boleh dianggarkan kerana zymogram analisis tidak memberi sebarang data.